IN THE CLAIMS:

In claim 13, line 19 of page 46, please delete the first occurrence of "at".

REMARKS

Entry of the above amendment and reconsideration of the present application are respectfully requested. Claims 1-30 are pending in the application, and have been rejected over various cited prior art. These rejections will be discussed below. The IDS has been cited as not complying with 37 C.F.R. §§1.97, 1.98 and MPEP § 609 as the reference was not translated. Applicants have obtained an English abstract of the reference for the Examiner's review. Applicants request that the Examiner consider the German reference on the merits.

Rejection of Claim 13 Under 35 U.S.C. § 112, Second Paragraph

The Examiner has rejected claim 13 because the word "at" is recited twice in the claim. Applicants thank the Examiner for noting this typographical error. Applicants have amended the claim appropriately and request that the rejection be withdrawn.

Claim Rejections Under 35 U.S.C. §§102(a) and 102(b)

Claims 1-13 stand rejected under 35 U.S.C. § 102(a) as being anticipated by Ho et al. (WO 95/13362). The recombinant yeasts referred to in the Ho et al. reference were created by cloning the three xylose metabolizing genes on a high-copy number plasmid, pUCKm10. The Ho et al. reference does not teach or suggest a yeast having genes integrated at each of multiple reiterated ribosomal

DNA sites of the yeast as recited in claims 1-13 of the present application. As these limitations are nowhere taught or suggested in the cited reference, this rejection is improper.

Claims 14-16, 18-19, 28 have also been rejected under 35 U.S.C. § 102(b) over Le Dall et al., and claims 14-16, 18-19, 28 and 30 have alternatively been rejected under 35 U.S.C. § 102(a) over Lopes et al. or under 35 U.S.C. § 102(b) over Fujii et al. Neither Le Dall et al., Fujii et al. or Lopes et al. teach or suggest a method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells by transforming the cells with a replicative and integrative plasmid and repeatedly replicating the cells to produce generations of progeny cells while maintaining the selection pressure, so as to promote retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA. Conversely, Le Dall et al., Lopes et al. and Fujii et al. teach methods that utilize plasmids that have been linearized for integration (YIp plasmids) that can not replicate in the yeast host cells utilized therein as they do not include a functional yeast DNA replication of origin. Withdrawal of the rejections under 35 U.S.C. §§102(a) and 102(b) is respectfully requested.

Rejection of Claims 103 Under 35 U.S.C. § 103(a)

Claims 1-30 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Yamano et al., in view of Le Dall et al., Fujii et al. and Tantirungkij et al. In reference to the method claims and plasmid claims of the

present application, Yamano et al. does not utilize a replicative plasmid that includes the exogenous DNA to be introduced into the cell. Yamano et al. teaches introduction of exogenous DNA, the Acetobacter ALDC gene, that is incorporated into a linearized plasmid, into brewer's yeast genome by co-transformation with pZNEO. The linearized plasmid that carries the ALDC gene into the cell does not contain an origin of replication that will allow the linearized plasmid to self-replicate in the yeast host.

Furthermore, as discussed above, Le Dall et al. and Fujii et al. do not utilize integrative plasmids having the ability to replicate in the host cells described therein. Moreover, Tantirungkij et al. utilized a vastly different method to increase the fermentation capacity of yeast. In previous work, Tantirungkij et al. transformed yeast with plasmid pEXGD8 that included the xylose reductase (XR) and xylose dehydrogenase (XD) genes. These transformants poorly fermented xylose to ethanol. In the currently cited reference, Tantirungkij et al. treated the transformed yeast with a chemical mutagen, methane sulfonate, in attempts to improve the xylose fermentation ability of the yeasts. It was unexpectedly found that one of the mutants contained XR and XD genes integrated into chromosomes XII or IV. Although chemical mutagenesis is a powerful tool, it is also an unpredictable and unreliable tool for genetically modifying an organism. It may be very difficult to duplicate what Tantirungkij et al. observed. Due to the unpredictable nature of mutagenesis, and the fact that Le Dall et al. and Fujii et al. do not utilize a replicative and integrative plasmid, not only would one skilled in the art not be motivated to combine these

references, there is also no reasonable chance of success in modifying Yamano et al. with Le Dall et al., Fujii et al., and/or Tantirungkij et al. to arrive at the claimed methods, yeasts and plasmids.

Furthermore, with respect to Yamano et al., Le Dall et al. and Fujii et al., the exogenous gene must be integrated into the chromosome immediately following transformation, or else it will be lost. In the methods recited in the present application, the integration process can take place at the onset of transformation and/or, in a majority of cases, continuously in a controlled manner after the process of transformation has long been completed as described on page 19 of the application. Yeasts, plasmids, and methods utilizing the plasmids of the present invention, as recited in the pending claims are exemplified by the data shown in FIGS. A-H.

Referring now to FIGS. A-D, the ability of yeast transformants, produced in accordance with the methods of the present invention, to ferment glucose and xylose as a function of time is shown. These figures, as well as FIGS. E-G discussed below, are true and accurate copies of figures provided to applicant's attorney by inventor Dr. Nancy W.Y. Ho. These figures are graphical representations of data obtained by Dr. Ho in experiments she performed to further characterize the yeasts and methods recited in the application. Referring to FIG. A, 259A(LNH-ST)(Int 0) is one of the initial transformants selected after transformation of yeast strain 259A with the plasmid pLNH-ST. Referring to FIGS. B-D, 259A(LNH-ST)(Int 1,-(Int 2), and –(Int 3) are derivatives of 259(LNH-ST)(Int 0) obtained after the latter had been cultured in a special integration

medium that promoted further integration of the integration cassette containing the three xylose metabolizing genes into the yeast chromosome. As can be seen by reviewing the figures, the longer 259(LNH-ST)(Int 0) was cultured in the integration medium, the more copies of the three xylose-metabolizing genes were integrated as shown by an increased rate of xylose fermentation. That is, 259(LNH-ST)(Int 3) includes more copies of the integrated three-gene cassette than -(Int 2), -(Int 2) includes more copies of the integrated cassette than -(Int 1), and -(Int 1) includes more copies of the integrated cassette than -(Int 0).

Moreover, the yeasts in accordance with the invention that include multiple integrated copies of XR, XD and xylulokinase (XK) are surprisingly stable with respect to their fermentation capacity, and such yeast are not taught or suggested in the cited prior art. These yeasts address specific needs in the yeast fermentation industry in providing highly stable fermentation of biomass, including glucose and xylose as primary sugars, without significant loss of fermentative capacity even after numerous generations of culture under non-selective conditions. For example, as directly claimed in claim 25, yeasts in accordance with this invention can substantially retain their capacity for fermenting xylose to ethanol when cultured under non-selective conditions for at least twenty generations. The Examiner's attention is drawn to the specific Examples, such as Example 7 and FIGS. 4-7, wherein it is clearly demonstrated that yeasts in accordance with the invention [1400(LNH-ST)] are surprisingly stable, retaining essentially all of their fermentative capacity after forty

generations (see FIG. 7) as compared to corresponding non-stable strains (see FIGS. 4-6). Moreover, the above results are also reproducibly obtained.

For example, referring now to FIG. E, it is observed that a newly-developed strain 259A(LNH-ST) in accordance with the invention is stable in that it can be cultured in non-selective medium for more than forty generations without losing its activity for cofermenting glucose and xlyose to ethanol. With respect to FIG. F, it is seen that another newly-developed strain 424A(LNH-ST) in accordance with the invention can repeatedly coferment glucose and xylose to ethanol and can perform this fermentation for more than twenty generations as seen in FIG. G and for more than forty generations without losing its cofermenting activity (data not shown). The above surprising results are nowhere taught, suggested or disclosed in the cited references. In fact, the cited references themselves teach the unpredictability and potential mitotic instability which occurs in transformed yeast. For example, Lopes et al, on page 472, expressly teaches the mitotic instability of its transformed yeasts, and similar teachings are found in other references.

There is no teaching or suggestion of the methods, plasmids or yeasts recited in the rejected claims in light of the above results relating to stability of the yeasts and the different and novel integration methods developed by the present inventor and there is no motivation to combine the above references for the reasons cited above.

Furthermore, none of the cited references combined teach or suggest a yeast which ferments xylose to ethanol that includes the XR, XD and XK genes

integrated at each of multiple reiterated ribosomal DNA sites of the yeast as recited in claims 1-13 of the present invention. Although Tantirungkij et al. unexpectedly found that one of their mutants contained XR and XD genes integrated into chromosomes XII or IV, it is realized that only two desired genes were incorporated. In regard to claims 1-13, yeasts are described that include a third gene encoding XK incorporated into its genome. The effect of an extra gene on the integration process can not be predicted.

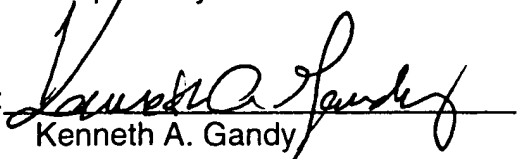
Referring now to the next rejection, claims 1-30 have further been rejected under 35 U.S.C. § 103(a) as being unpatentable over Ho et al. (WO 95/13362), in view of Le Dall et al. and Fujii et al. None of these references teach or suggest a yeast which includes the three xylose-metabolizing genes integrated at each of multiple reiterated ribosomal DNA sites of the yeast. For example, Ho et al. teaches cloning of the three xylose-metabolizing genes, XR, XD and XK, all fused to promoters on a 2 μ-based high copy number plasmid. This methodology was not effective to integrate the three xylose-metabolizing genes into the yeast chromosome under controlled conditions to insure an optimal copy number for strong activity for co-fermenting glucose and xylose to ethanol while not necessarily burdening the host cell. The stability of the cloned genes in the present invention, as shown and described above in FIG. 7, is improved relative to the cloned genes of Ho et al. (W095/13362), as seen in FIGS. 5 and 6. There is absolutely no teaching or suggestion of forming yeasts that have therein integrated the xylose-metabolizing genes in reiterated multiple reiterated ribosomal sites, nor is there any teaching or suggestion of utilizing the replicative

and integrative plasmids in a method for integration or for forming cells having multiple integrated copies of an exogenous DNA fragment.

Le Dall et al. and Fujii et al. have been described above and add no further incentive to arrive at the claimed invention. As described above, the linearized integrative plasmids of Le Dall et al. and Fujii et al. could not be used in the methods of the present invention wherein it is necessary to repeatedly replicate transformed cells so as to promote retention of a replicative and integrative plasmid in subsequent generations of the progeny. Thus, there would be no reasonable chance of success in placing the xylose-metabolizing genes of Ho et al. into the ribosomal vector of Le Dall et al. and Fujii et al. in order to integrate multiple copies of the genes into reiterated chromosomal DNA of the yeast according to the methods claimed in the present application. Withdrawal of the rejections of claims 1-30 under 35 U.S.C. § 103(a) is respectfully requested.

In view of the foregoing, it is submitted that the present application is in condition for allowance. Action toward this end is solicited.

Respectfully submitted

By: 

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